on the viral envelope have been seen in unrelated viruses (e.g., flaviviruses [Mukhopadhyay et al., 2005]). Although such 2D networks have not been identified on the envelopes of herpesviruses, cryoelectron tomography images of HSV-1 show local clustering of viral proteins (Grunewald et al., 2003). Perhaps a similar imaging of EBV virions will reveal ordered envelope glycoproteins and confirm this interesting hypothesis.

From these and previous data, one could derive the following model (Figure 1B). On the viral surface, gp42 is in complex with gH/gL, bound to residues 36–81; the linker connecting the gH/gL-binding site to the CTLD, residues 87–93, is bound to the HLA-binding site of a neighboring molecule. When HLA binds gp42, it would displace residues 87–93 and cause the hydrophobic pocket to widen. The changed conformation of residues 87–93 would then force the bound gH/gL to relocate. At the same time, the wider hydrophobic pocket could accommodate either gH/gL or gB. Although this model is largely speculative, it suggests new directions for probing the complex mechanism of EBV entry.

The authors compare this potential mechanism to that of HSV-1 gD. They note that just as receptor binding releases the HSV-1 gD C terminus, HLA binding releases N-terminal residues 87–93 of EBV gp42. The similarities may end here, however, because the triggering mechanism of gp42 has many differences with that of HSV-1 gD. For example, a stable gD/gH/gL complex has not yet been observed, but gp42 forms a ternary complex with gH/gL even in the absence of its receptor. Furthermore, although receptor binding frees the gD C terminus to interact with gH/gL or gB or both, residues 87–93 of gp42 are not involved in binding gH/gL. This region may act more like a hinge to reposition the gH/gL complex that remains bound upstream. More work is necessary to reveal the detailed mechanism of triggering in both EBV and HSV-1. For now, all that is certain is that the triggering of membrane fusion during the entry of EBV and HSV-1 shows more differences than similarities.

Although gp42 is critical for infection of B cells, it is dispensable for EBV entry into epithelial cells, which lack HLA. Indeed, several other herpesviruses do not require a specialized receptor-binding protein to trigger fusion (Heldwein and Krummenacher, 2008). So perhaps the real mystery is why in certain herpesviruses these specialized receptor-binding proteins are needed at all.

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For Structural Biology, Try Infrared Instead

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1D and 2D-IR spectroscopy was used to resolve the structural transition that is responsible for the gating mechanism of the influenza A M2 channel (Manor et al., 2009). This report constitutes a milestone in the development of both ATR-FTIR and 2D-IR spectroscopies as precise tools for structural biology.

Influenza A M2 proton channel is a homotetrameric integral membrane protein that plays an important role in the influenza A virus life cycle. Functionally, M2 protien is a pH-gated proton (H^+) channel, and details of the gating mechanism are not fully understood. In this issue, Manor et al. (2009) use a combination of sophisticated new vibrational spectroscopy methods, in combination with isotope labeling, to tackle and solve this long-standing problem of great biological relevance (i.e., the structural change that is responsible for the gating mechanism of the influenza A M2 channel [see Figure 4 of Manor et al. (2009)]). They show that the transmembrane helices turn by about 100° when switching from the closed to the

open state in response to a pH change. This finding will certainly help in the development of anti-flu agents that block its H^+ channel activity. At least as important, however, is the fact that the paper constitutes a milestone in the development of both attenuated total reflection (ATR) and two-dimensional infrared (2D-IR) spectroscopies, tools that have been underutilized in the structural biology community but hold tremendous promise. In the following, I shall briefly discuss why that is so.

Twenty years ago, vibrational spectroscopy was a pivotal technique used in structural biology, but it became overshadowed by NMR spectroscopy and X-ray scattering, largely because vibrational spectra of proteins are too congested to resolve individual residues and infrared technology was rudimentary. The advances in NMR and X-ray spectroscopy over the last few decades are, to a large extent, responsible for the great success of Francis Crick's approach: "If you want to understand function, study structure." Other methods, in particular fluorescence and EPR spectroscopy, although important and used frequently to study systems or functional aspects not easily assessable by NMR and X-ray spectroscopy, require special labels that can be relatively bulky or are chemically reactive, and hence introduce some degree of perturbations that might interfere with function.

With the advent of 2D-IR spectroscopy (Hamm et al., 1998), vibrational spectroscopy is now in a position to regain a place of importance in structural biology. An important prerequisite is isotope labeling, which may resolve a single C = O group (Arkin, 2006). As the authors show, attenuated total reflection Fourier transform IR (ATR-FTIR) spectroscopy can then be used to extract the angles of the labeled carbonyl groups relative to the membrane. ATR-FTIR spectroscopopy measures an IR spectrum of membranes layers attached to a surface that therefore are oriented, and, by using polarized light, angle information can be extracted. 2D-IR spectroscopy, on the other hand, was used to characterize the local environment of the labeled carbonyl group and its secondary structure. The result is structural resolution comparable to solid-state NMR and X-ray crystallography without the worry of the impact of isotope labels on altering the structure. The agreement of the ATR-FTIR derived angles with the solid-state NMR and X-ray structures is amazing when the simplicity of the ATR-FTIR technique is considered. As for 2D-IR spectroscopy, this paper is a milestone because it demonstrates that 2D-IR spectroscopy has now matured into a method that is capable of answering very relevant questions in structural biology for which established methods might not hold the answers.

2D-IR spectroscopy utilizes many concepts of 2D-NMR spectroscopy and applies them to vibrational transitions. That is, 2D-IR spectroscopy investigates the vibrations of certain molecular groups (in most cases the C = O groups of the protein backbone) rather than nuclear spins (see Cho [2006], Zheng et al. [2007], Hamm et al. [2008] and Ganim et al. [2008] for recent review articles on 2D-IR spectroscopy from various perspectives). Just like in NMR or EPR spectroscopy, the vibrations of the C = O groups are not really the object of interest; rather, they are used as molecular spies that respond in some way to the immediate environment. In the particular example (Manor et al., 2009), it is the spectral broadening of the C = O vibrations that reports on the larger polarity inside the ion channel pore, as well as on its inhomogeneity. This is the essential information content that Manor et al. (2009) used to construct a structural model of the channel. The polarity of the pore is also crucial for its function. The relationship between infrared spectral width and polarity is currently largely empirical, but theory is being established so that it may serve as a more quantitative structural indicator. The explanatory power of NMR spectroscopy builds on a huge toolbox of such empirical rules, and by comparison, 2D-IR spectroscopy is in its infancy. However, as more papers in the nature of Manor et al. (2009) are published, the 2D-IR toolbox will further grow.

One unique feature of vibrational spectroscopy is its intrinsically fast time-resolution, which is on the order of 100 fs to 1 ps. Molecular systems are essentially frozen on the infrared timescale. In other words, vibrational spectroscopy measures a quasistatic snapshot of a distribution of local structures around the molecular spy, whereas both NMR and EPR spectroscopy time-average over these structures for a period of milliseconds or microseconds, respectively. It is this fast time-resolution that enabled Manor et al. (2009) to extract the informative structural information (i.e., the inhomogeneity of the polarity inside the ion channel pore *versus* that in the hydrophobic environment of the membrane).

Another potential aspect of the high intrinsic time-resolution of vibrational spectroscopy has not even been touched yet by Manor et al. (2009). Vibrational spectroscopy might resolve the structural transition of the channel in a time-resolved manner with essentially unlimited time-resolution (Kolano et al., 2006; Strasfeld et al., 2008) after, for example, a laser-induced pH jump (Causgrove and Dyer, 2006). Hence, vibrational spectroscopy holds the promise to become even more important in the post structure-function-relationship era; the notion that it is not only structure that is responsible of biological function, but the dynamics of structures. The paper of Manor et al. (2009) constitutes an important step in this direction.

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